Functional characterization of ZiP8, a zinc transporter with potential relevance for neuropsychiatric disorders

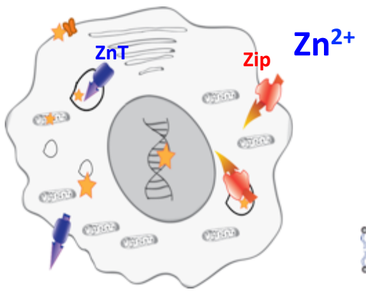
L. MARGER[1], D. BERTRAND[1], I. SINGEC[2], H. S. XI[3], J. R. WENDLAND[4], *C. R. SCHUBERT[4]

[1] HiQscreen, Geneva, Switzerland; [2] Neuroscience Research Unit, [3] Computational Sciences CoE, [4] PTx Clinical Research, Pfizer Inc., Cambridge, MA

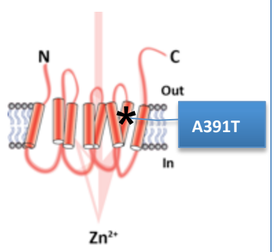
ABSTRACT

While the biological roles of some divalent cations (e.g. calcium, magnesium) are well-established, the basic biology of other trace elements such as zinc is still poorly understood. The importance of zinc can be exemplified by numerous zinc-dependent enzymes and DNA-binding of transcription factors depending on zinc finger motifs. In neurobiology, zinc has been implicated in the modulation of neurotransmission by interacting with glycine receptors almost two decades ago (Laube et al., 1995). Zinc is accumulated in specific CNS neurons and can be released during synaptic activity and has been studied in the context of some neurological disorders such as Wilson's disease, Pick's disease or epilepsy (Assaf and Chung, 1984). More recently, important progress was made in identifying zinc transporters that are expressed in many different neural and non-neural cell types. Currently, at least twenty-four distinct transporter proteins have been identified and ongoing work is aimed at characterizing these transporters. The two main classes of zinc transporters, the ZnT and ZiP families, are specifically expressed in different cell types and subcellular compartments. It is thought that ZnT can decrease intracellular zinc concentration, whereas ZiP regulates zinc homeostasis by actively transporting zinc cations across the cell membrane. Interestingly, several disorders are associated with zinc transport dysfunction including osteoarthritis (Kim et al., 2014) and neurological disorders with cognitive deficits such as autism and schizophrenia (Grabrucker et al., 2014; Takeda and Tamano, 2014). In the present work, we examined the functional properties of the plasma membrane transporter ZiP8 and its ability to transport zinc in various cellular model systems in the presence of a specific point mutation (A391T). Heterologous expression of normal and mutated ZiP8 in *Xenopus* oocytes and mammalian cells demonstrated significantly altered zinc transport due to the A391T mutation. We are currently validating these findings in human neurons derived from embryonic stem cells with the ultimate goal to establish a large-scale "humanized" screening assay for drug discovery.

Cellular localization of zinc transporter:



Structure of ZiP8:



FLUORESCENCE IMAGING

Figure 1. Zinquin absorption and emission spectra

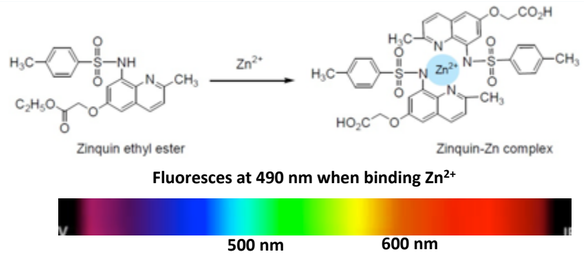
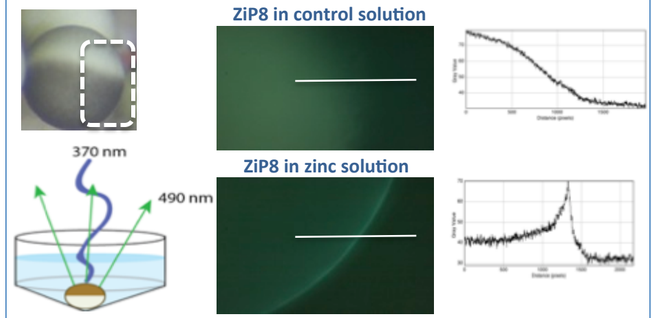


Figure 2. Zinc transport in oocytes revealed by Zinquin

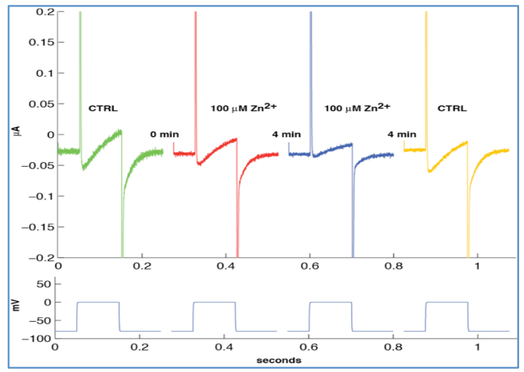


Results obtained in a series of oocytes injected with ZiP8, loaded with Zinquin panel up and loaded with Zinquin and incubated in presence of 100 μM zinc and 25 mM $NaHCO_3$ panel down. The graphics represent the pixel intensity as a function of distance measured along the white bar.

USE OF A REPORTER CHANNEL TO DETECT INTRACELLULAR ZINC

Figure 4. Zinc transport monitored by Ca_V 1.2

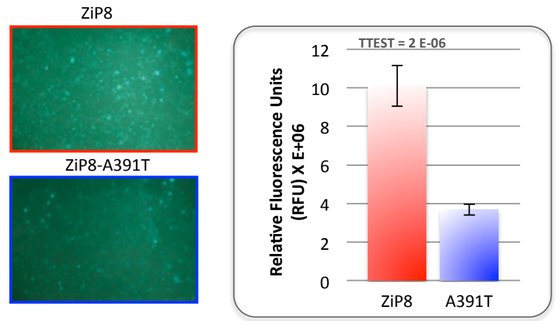
Zinc inhibits Ca_V1.2 in the nanomolar range

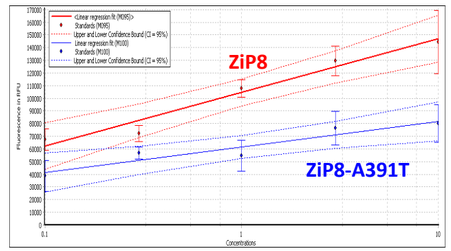


A voltage dependent calcium channel (Ca_V1.2) is used to monitor ZiP8 activity in oocytes. These traces show the amplitude of the I_{CaL} is reduced upon exposure to extracellular zinc (100 μM).

MUTATION A391T ALTERS ZINC TRANSPORT

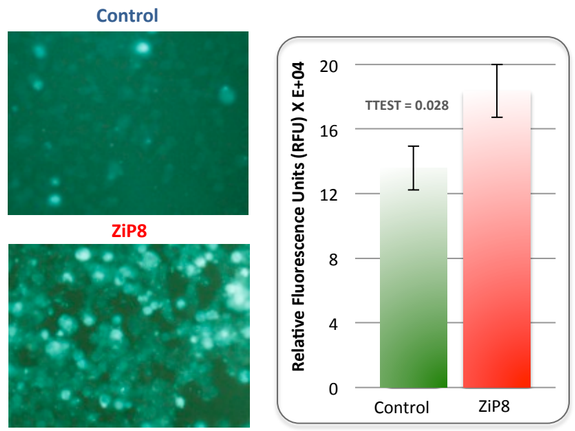
Figure 5. ZiP8-A391T displays a lower zinc transport activity



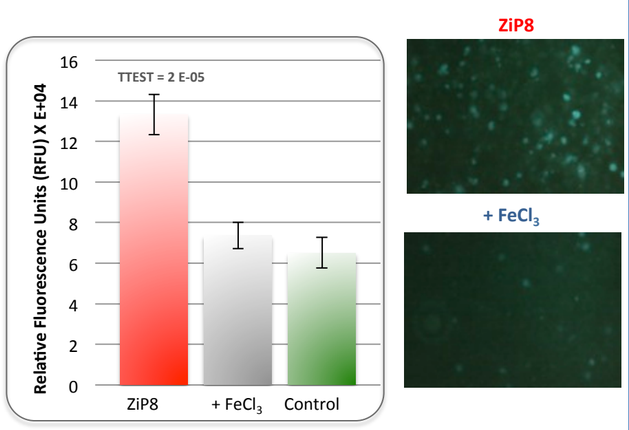


Fluorescence readout of Zinquin was measured in ZiP8 and ZiP8-A391T transfected cells at different external zinc concentrations using a microplate reader (n=16).

Figure 3. Zinquin fluorescence in HEK-293 cells expressing ZiP8



Images of cells from the same batch, not-transfected (Control) or transfected with ZiP8 and incubated with 20 μM Zinquin, captured after incubation for 30 minutes in presence of 100 μM zinc.



Fluorescence readout conducted in 96 well microtiter plate showed a reduction of fluorescence in cells incubated in presence of zinc (100 μM) and $FeCl_3$ (1 mM).

CONCLUSION

Expression of ZiP8 was successfully obtained in HEK-293 cells and in *Xenopus* oocytes. Quantification of zinc transport using a fluorescent probe or a channel reporter revealed the fast kinetics of intracellular zinc increase both in cells and *Xenopus* oocytes. Characterization of the ZiP8 mutant that is associated with schizophrenia unveiled a diminished activity, which could be at the origin of the disease.

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